DI-N-HEXYL PHTHALATE

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1.0 EXPOSURE

1.1 Chemistry

Di-n-hexyl phthalate (DnHP) (CAS number 84-75-3) is produced by reacting phthalic anhydride and normal hexyl alcohol in the presence of an acid catalyst (CMA 1999). DnHP is often found as a minor component (less than 1%) of 6,10- phthalate mixtures; it may also be an isomer in mixtures of diisohexyl phthalates (DIHP) (CAS number 68515-50-4) at levels of 25% or less (*2*).

Synonyms: 84-75-3 -- 1,2-Benzenedicarboxylic acid, dihexyl ester; dihexyl ester phthalic acid; di-n-hexyl phthalate; DnHP)

68515-50-4 -- 1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear; diisohexyl phthalate; DIHP

Table 1: Properties of DHP (isomer not clearly identified)

Property	**Value**
Chemical Formula	$C_{20}H_{30}04$
Molecular Weight	334.4
Vapor Pressure	5×10^{-6} mmHg at 25 ^{o}C
Melting Point	- 27.4 ^{o}C
Boiling Point	350 ^{o}C
Specific Gravity	1.011
Solubility in Water	Slight – 0.05 mg/L
Log K_{ow}	6.3

(*10*)

1.2 Exposure

Exposure to DnHP can occur from three sources: migration from consumer products where it has limited use; its presence as a minor component (less than 1%) of commercial 6,10-phthalates; and as a component of commercial diisohexyl phthalate (DIHP) where it may attain concentrations of up to 25%. DnHP is used in the making of plastisols that are subsequently used in the manufacture of automobile parts (air filters, battery covers) and dip molded products (tool handles, dishwasher baskets) (*1*). Commercial phthalate substances containing DnHP may be used in PVC utilized in the manufacture of flooring, canvas tarps, and notebook covers (*2*). Phthalates substances containing DnHP may also be used in traffic cones, toys, vinyl gloves, weather stripping, flea collars, shoes, and conveyor belts used in food packaging operations.

There is currently no information on production volumes of DnHP but production is stated to be “small” compared to other phthalates. Limited information is available for production volumes or consumption rates of 6,10-phthalate and DIHP. About 25 thousand tons of 6,10-phthalate were produced in the United States in 1994 (*3*). The DnHP content would equal less than 250 tons. The annual consumption rate of DIHP in Europe was reported as less than 2,000 tons (*4*) therefore, DnHP consumption could be 500 tons.

Release of DnHP to the environment can occur during the production of DnHP, 6,10-phthalates, or DIHP, and during the incorporation of the phthalates into plastic resins. Because DnHP, like other phthalates, is not bound to plastics, it can be released during the use or disposal of the product. Phthalates that are released to the environment can be deposited on or taken up by crops that are intended for human or livestock consumption, and thus enter the food supply.

General Population Exposure

The general population may be exposed to phthalates primarily through the oral and dermal routes. Based on data for other phthalates, the most likely source of human exposure to DnHP is dietary intake. DnHP may be found in food as a result of environmental uptake during cultivation or as a result of migration from processing equipment or packaging materials. In a survey of packaged fatty foods purchased from grocery stores in the UK, DHP (isomer not specified) was detected, but not quantified, in carcass meat, poultry, eggs, and milk (*5*). DHP (isomer not specified) was detected but not quantified in 7/12 baby formulas from the UK (*6*). DHP levels in infant formula were not reported in an MAFF follow-up analysis (*7*).

Mouthing of toys is a potential source of oral phthalate exposure in children. There were no studies identified that documented the detection of DnHP-containing compounds in children's toys.

Dermal contact with products containing DnHP is possible, but absorption through skin is unlikely. Studies in rats have demonstrated that DnHP is poorly absorbed through skin (Elsisi et al. 1989). An *in vitro* study conducted with other phthalates suggests that the DnHP absorption rate for human skin is lower than the absorption rate for rat skin (*8*).

The available data do not allow the estimation of DnHP exposures to the general population. However, a comparison of production volumes and consumption rates for DnHP-containing compounds versus those containing DEHP, suggests that human exposure to DnHP is well below the exposure value for DEHP, which was estimated at 3–30 μg/kg bw/day by Doull et al. (*9*) (see also DEHP monograph).

Medical Exposure

There are no known uses of DnHP or DnHP-containing compounds in medical devices.

Occupational Exposure

Workers may be exposed to DnHP primarily through inhalation and dermal contact. Phthalates are manufactured within closed systems, but exposure to workers can occur during filtering or loading/unloading of tankcars (*2*). Higher exposures to phthalates can occur during the production of flexible PVC because the processes are open and run at higher temperatures. According to the CMA (*2*), phthalate levels in air are generally less than 1 mg/m^3 and 2 mg/m^3 during the production of phthalates and flexible PVC, respectively. Exposure levels were estimated by the CMA (*2*) using assumptions of a 10 m^3/day inhalation rate and a 70 kg body weight. The resulting exposure estimates were 143 μg/kg bw/day and 286 μg/kg bw/day for workers employed in phthalate and flexible PVC manufacturing operations, respectively. If the total number of days worked per year is assumed to be 220 days, the exposure estimates convert to 86 and 172 μg/kg bw/day.

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 General Toxicity

2.1.1 Human Data

There were no human data identified.

2.1.2 Experimental Animal Data

Systemic effects following DnHP treatment for either 3, 10, or 21 days were examined in 4-week-old Wistar rats (*11*). The effects were compared to those produced by approximately equal concentrations of DnOP, another straight-chained phthalate, and DEHP, a branched-chained phthalate (*11*). A group of 12 male rats was a fed a diet containing 20,000 ppm DnHP and a control group of 18 rats was fed the basal diet. Using actual food intake levels and rat body weights on the day of sacrifice, a DnHP dose of 1,824 mg/kg bw/day was calculated. Groups of 4 treated rats and 6 control rats were sacrificed and necropsied after 3, 10, and 21 days of treatment. Liver histopathology, enzyme activity, and peroxisome proliferation were examined. Levels of thyroid hormones in serum and thyroid histopathology were also examined (*12*).

DnHP treatment did not cause a change in body weight gain or food intake levels. DnHP treatment had no effect on testes weight or the gross appearance of testes, kidney, or pancreas (*11*). However, liver weight was significantly increased following 21 days of DnHP treatment, with histology and chemistry changes observed at all 3 assessment times. Centrilobular necrosis and loss of glycogen were first observed at 3 days and centrilobular fatty accumulation was observed at 10 days of treatment. The effects became more pronounced with increasing duration of treatment. Examination by electron microscopy revealed proliferation and dilation of smooth endoplasmic reticuli and shortening of the microvilli in bile canaliculi at 3 days, the presence of lipid droplets within hepatocytes at 10 days, and possibly a small increase in lysosomes and peroxisomes at 3 and 21 days, respectively. The activity of the peroxisomal proliferation marker, cyanide-insensitive palmitoyl CoA oxidase, was significantly increased at levels approximately 2-fold greater than controls in rats only after 10 days of treatment. There was no change in total catalase activity, but catalase activity in the particulate fraction was significantly increased at 10 and 21 days of treatment. A significant decrease in glucose-6-phosphate activity at 21 days of treatment was the only other effect on liver enzymes. Effects of DnOP, DnHP, and DEHP on thyroid were studied in rats. Each phthalate was associated with a decrease in serum thyroxine (T4) levels; serum triiodothyronine (T3) levels were essentially unaffected. Electron microscopic changes indicative of thyroid hyperactivity (increased lysosomal numbers and size, enlarged Golgi apparatus, and mitochondrial damage) were also observed (*12*).

In a comparison of the three tested phthalates, the effects induced by DnHP were similar to DnOP but different from DEHP. DEHP treatment resulted in a more pronounced increase in liver weight and in increased mitotic activity. Less fat accumulated following treatment with DEHP, and when observed, the accumulation occurred in the midzonal and periportal zones rather than in the centrilobular region. Biochemical evidence of peroxisomal proliferation (cyanide-insensitive palmitoyl CoA oxidation) occurred earlier with DEHP treatment (after 3 days of treatment) and was approximately 7-fold higher than it was following DnHP or DnOP treatment. Although DEHP was a stronger inducer of peroxisome proliferation, DnHP and DnOP also induced peroxisome proliferation following longer treatment periods. Other effects suggesting liver damage were also observed following DnHP treatment. Thyroid effects were similar for all three phthalates.

Table 2: Summary of Changes in the Livers of Rats Administered Diets Containing 2% w/w DEHP, DnOP, or DnHP

EFFECT	Treatment		
	DEHP	DnHP	DnOP
Liver Morphology			
Hepatomegaly	+++	+(Late)	+(Late)
Centrilobular loss glycogen	+	+	++
Centrilobular necrosis	–	++	++
Peroxisome proliferation	+++	+(Late)	+(Late)
Smooth endoplasmic proliferation	++	+	+
Increase of inner mitochondrial matrix	++	–	–
Initial burst of mitosis	++	–	–
Liver Biochemistry (d21)			
Cyanide-insensitive palmitoyl CoA oxidation day 10	↑↑↑↑	↑	↑
α–Glycerophosphate dehydrogenase day 21	↑↑	–	–
G-6-Phosphate day 21	↓↓↓	↓	↓
Succinate dehydrogenase μmol/min/g Protein day 21	–	–	↓
Catalase day 10	↓	↑↑	↑↑
Thyroid function			
Serum triiodothyronine (T_3) day 21	140%[c]	183%	133%
Serum thyroxine (T_4) day 21	64%	58%	76%

Adapted from (*11, 12*)
[a] (+) denotes the degree of change seen when compared to age-matched controls.
[b](–) = absence of the lesion or effect.
[c] Expressed as % of control value.

Summary

See Section 5.1.2 for summary of general toxicity effects

2.2 Toxicokinetics

Phthalate Associated Toxicokinetics

Absorption

Rodents

Dermal

Dermal absorption of DnHP has been studied along with a series of phthalates in the rat (*13*). Hair from a skin area (1.3 cm in diameter) on the back of male F344 rats was clipped, the ^{14}C-phthalate diester was applied in a dose of 157 µmol/kg, and the area of application was covered with a perforated cap. The rats were restrained and housed for 7 days in a metabolic cage that allowed separate collection of urine and feces. Urine and feces were collected every 24 hours, and the amount of ^{14}C excreted was taken as an index of the percutaneous absorption. At 24 hours, diethyl phthalate showed the greatest excretion (26%). As the length of the alkyl side chain increased, the amount of ^{14}C excreted in the first 24 hours decreased significantly. The cumulative percentage dose excreted in 7 days was greatest for diethyl, dibutyl, and diisobutyl phthalate, about 50–60% of the applied ^{14}C; and intermediate (20–40%) for dimethyl, benzyl butyl, and dihexyl phthalate (DnHP excretion was approximately 18%). Urine was the major route of excretion of all phthalate diesters except for diisodecyl phthalate. This compound was poorly absorbed and showed almost no urinary excretion. After 7 days, the percentage dose for each phthalate that remained in the body was minimal and showed no specific tissue distribution. Most of the unexcreted dose remained in the area of application. These data show that the structure of the phthalate diester determines the degree of dermal absorption. Absorption maximized with diethyl phthalate and then decreased significantly as the alkyl side chain length increased. Urine was the principal route of excretion, and there was no evidence of accumulation in any tissue.

Biotransformation

No oral or inhalation toxicokinetic data have been reported for DnHP. However, as other phthalates are converted to monoesters and rapidly excreted, it is anticipated that dihexyl phthalate would behave in the same way.

Distribution

Dermally absorbed DnHP was widely distributed throughout the body with no tissue containing >0.6% of the applied dose. There was no evidence for accumulation in any tissue.

Excretion

The major route of excretion of dermally absorbed DnHP was via the urine (*13*).

2.3 Genetic Toxicity

In genetic toxicity tests DnHP was inactive in the Salmonella/mammalian microsome mutagenicity assay with and without activation (*14*). According to a review conducted by the CMA (*2*), DnHP also tested negative in two bacterial assays. Barber et al. (*15*) tested 6,10-phthalate, which contains minor amounts of DnHP, in the mouse lymphoma mutation and Balb/3T3 cell transformation assays. Negative results were obtained in the cell transformation assay, but results of the mouse lymphoma mutation assay were considered equivocal due to the non-dose related increase in mutation frequency in non-activated cells.

The CMA (*2*) has reported that DIHP (which may contain up to 25% DnHP) was inactive in a mouse micronucleus test.

2.4 Summary

The summary for Section 2 is located in Section 5.1.2.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

Human data were not located.

3.2 Experimental Animal Toxicity

DnHP (CAS No. 84-75-3) was evaluated in the Chernoff-Kavlock screening assay (*16*). CD-1 mice (48–50 dams/group) were gavaged on gd 6–13 with 9,900 mg DnHP/kg/day (undiluted chemical, 10 mL/kg/day) or the corn oil vehicle. Dams were allowed to deliver their litters and both dams and pups were terminated on pnd 3. One exposed dam died. There were no live litters (0/34).

Nine other phthalates were evaluated in the Chernoff-Kavlock screening assay and the authors concluded that "dramatically positive results were seen with the diesters having intermediate chain lengths: n-butyl, i-butyl, and n-hexyl. The shorter (methyl and ethyl) and longer (n-octyl and i-decyl) diesters were generally negative, although litter size and neonatal weight gain were both reduced in the di (n-octyl) phthalate group relative to its concurrent control" (*16*).

A limited number of developmental effects were observed in a continuous breeding study that exposed CD-1 mice to dietary DnHP concentrations of 0, 0.3, 0.6, or 1.2% (0, 380, 800, or 1,670 mg/kg bw/day) (*17*, *18*). Complete details of this study are included in Section 4. Developmental effects could not be evaluated at the top two doses due to either very high rates of infertility or complete infertility. The number of live pups/litter was reduced in the 380 mg/kg bw/day group (n= 3 versus 12 in control group).

3.3 Summary

The summary for developmental toxicity is located in Section 5.1.3.

4.0 REPRODUCTIVE TOXICITY

4.1 Human Data

Human data were not located.

4.2 Experimental Animal Data

Two studies were reviewed in the evaluation of the reproductive toxicity of DnHP. None of the studies available are considered definitive and no multigeneration reproduction study has been published for this phthalate ester. Only one study measures effects of the agent on reproductive function in the mouse. The other has shown subacute effects of DnHP on testicular weight and morphology at high dose levels in the rat.

The key study for the assessment of the reproductive toxicity of DnHP is reported by Lamb et al. (*19*) and Reel et al. (*18*). In Lamb et al. (*19*) (Table WEB-1), DnHP was one of four phthalate esters compared using the Continuous Breeding protocol in mice (see DBP above). Twenty pairs of male and female CD-1 mice (40 pairs in control group) were dosed with DnHP for 7 days prior to and during a 98-day cohabitation period. The doses were 0, 0.3, 0.6, or 1.2% w/w in the diet. Intake levels in mg/kg bw/day were not reported in the original study by Reel et al. (*18*) or in the summary by Lamb et al. (*19*). However, intakes were estimated in other summaries of RACB studies. Morrissey et al. (*20*) estimated intake levels of 0, 430, 880, or 1,870 mg/kg bw/day and Chapin and Sloane (*21*) estimated intake levels of 0, 380, 800, or 1,670 mg/kg bw/day. The Expert Panel noted that the differences in estimated doses were small and biologically insignificant considering the inherent variability in such estimates. This variability reflects dramatic changes in animal weight during the study, which is common as the animal matures. For consistency, the values of Chapin and Sloane will be used throughout this monograph. Litters were examined and removed. Reproductive function was evaluated during the cohabitation period by measuring the numbers of litters per pair, number of live pups per litter, pup weight, and offspring survival. Organs were collected for histological evaluation and testes were preserved in Bouin's solution. DnHP exposure resulted in a dose-related reduction in the proportion of pairs able to produce even a single litter during the continuous breeding phase. No litters were produced at the high dose (1,670 mg/kg bw/day), 1 litter in the mid-dose group (800 mg/kg bw/day), 14 of 17 pairs had litters in the low-dose group (380 mg/kg bw/day), compared to all pairs having litters in the control group. The numbers of litters per pair, the number of live pups per litter, and the proportion of pups born alive were also significantly affected by DnHP exposure. Significant effects occurred at the lowest dose level with clear adverse effects seen in the absence of any body weight effects.

A crossover mating trial was performed between the high-dose males and control females. There was a significant decrease in detected matings (56%) compared to controls (90%), and only 1 of 18 treated males sired a litter. When the high-dose females were mated with control males, there was no decrease in copulatory plugs, but none of the females became pregnant. Only the control and high-dose DnHP groups were necropsied. Sperm assessment showed a significant decrease in sperm number (7% of control) and motility (22% of control) parameters. Only 3 of 18 males had sufficient numbers of sperm to allow assessment of abnormal forms; incidence in these 3 was diminished in number compared to control. There were significant decreases in the relative weights of the epididymis, testis, and seminal vesicles. There was extensive atrophy of the seminiferous epithelium with mature sperm markedly diminished in the epididymis. No treatment-related microscopic lesions were detected in the ovaries, uterus, or vagina of the female mice. For females, liver to body weight ratio was significantly increased (31%) and uterine weight significantly decreased (31%). Body and relative kidney/adrenal weights were significantly decreased and liver to body weight ratio was significantly increased in both males and females of the high-dose group, but histological changes were not noted. A second generation was not evaluated.

In a short-term study (*22*) which employed a single dose level of DnHP (2.4 g/kg bw/day) given by gavage in corn oil to a group of 12 pubertal male Sprague Dawley rats (4 weeks of age) for 4 days, marked effects on testis weight (65% of control value) were noted in the absence of body weight effects. Histologic examination of formalin-preserved testes revealed a marked seminiferous tubular atrophy with the majority of tubules showing few spermatogonia and Sertoli cells, but normal Leydig cell morphology.

Mode of Action

DnHP has been studied in an *in vitro* assay in order determine the mechanism of testicular toxicity. Incubation of Sertoli and germ cell cultures with 1, 10, or 100 μM DnHP resulted in a dose-related detachment of germ cells from the Sertoli cell monolayer (*23*). The detached germ cells were viable and structurally normal, but changes were observed in the morphology of the Sertoli cells. The findings suggest that germ cell loss following *in vivo* exposure to DnHP is a secondary effect resulting from toxic insult to Sertoli cells.

The estrogenic activity of DIHP has been examined using a battery of short-term *in vitro* and *in vivo* assays. DIHP did compete with tritiated estradiol for binding to the rat uterine cytosolic estrogen receptor (*24*). DIHP, in contrast to the positive control estradiol, did not significantly induce an *in vivo* vaginal cornification response or increase in uterine weight at any of the concentrations tested (20, 200, and 2,000 mg/kg) over the course of a 5-day experiment using immature and adult ovariectomized Sprague Dawley rats (*24*). DnHP is a constituent of DIHP and may reach concentrations of 25% of the complex substance.

4.3 Summary

The summary for reproductive toxicity is located in Section 5.1.4.

5.0 DATA SUMMARY & INTEGRATION

5.1 Summary

5.1.1 Human Exposure

A limited quantity of DnHP is produced for commercial use in automotive parts and dip molded products such as dishwasher baskets and tool handles (*1*). It is a component of other phthalate mixtures and may constitute up to 25% of commercial diisohexyl phthalates (DIHP). The compound is also present in 6,10-phthalate substances at less than 1% concentration. Assuming equivalent production and usage of 6,10-phthalates and DIHP in the US and Europe, the aggregate annual production of DnHP in these two products could be as much as 750 tons. Phthalates containing DnHP may be used in PVC used to manufacture flooring, canvas tarps, and notebook covers (*2*). Such phthalates may also be used in traffic cones, toys, vinyl gloves, weather stripping, flea collars, shoes, and conveyor belts used in food packaging operations.

DHP (isomer not specified) was detected but not quantified in 7 of 12 baby formulas from the UK (*6*). DHP levels in infant formula were not reported in a MAFF follow-up analysis (*7*). In a survey of packaged fatty foods purchased from grocery stores in the UK, DHP (isomer not specified) was detected, but not quantified, in carcass meat, poultry, eggs, and milk (*5*). Based on production volumes and consumption rates of DnHP-containing compounds versus DEHP, human exposure to DnHP is likely lower than exposure to DEHP, which was estimated at 3–30 μg/kg bw/day by Doull et al. (*9*). In occupational settings, exposure is thought to be highest in workers of flexible PVC manufacturing facilities. Based on general levels of phthalates reported, the CMA (*2*) estimated an exposure level of 286 μg/kg bw/day with an average yearly exposure of 172 μg/kg bw/day.

Utility of the Data for CERHR Evaluation. There is very limited information on exposure and exposure pathways to DnHP in humans. Such estimates are complicated as DnHP is rarely produced directly for commercial use but is a component (up to 25%) in commercial diisohexyl phthalates (DIHP) and at less than 1% in 6,10- phthalate substances. 6,10- Phthalates and DIHP are used in a variety of consumer products. DnHP has been detected in environmental samples (air, water, and soil); however, quantitative estimates for exposure are limited.

5.1.2 General Biological and Toxicological Data

Human data were not found for the categories presented in this section.

General Toxicity. General toxicity information for DnHP is limited but is available from a repeated-dose dietary study in which 4 Wistar rats (4-weeks old) were exposed to 1 high dose (1,824 mg/kg bw/day) for 3, 10, or 21 days (*11*, *12*). The liver was identified as the principal target organ and effects observed included necrosis, fatty accumulation, and glycogen loss. DnHP was found to be a weak peroxisome proliferator as evidenced by both morphological and biochemical enzyme profiles compared to DEHP as effects occurred at later time points and to a lesser extent than with DEHP. Histologic changes in the thyroid suggested hyperactivity as evidenced by microscopic changes.

Toxicokinetics. No oral or inhalation toxicokinetic data have been reported for DnHP. DnHP is absorbed dermally in rats and is excreted via urine, with approximately 18% of ^{14}C excreted within 7 days. After 7 days, the percentage that remained in the body was minimal based on a study by Elsisi et al. (*13*), and showed no specific tissue distribution. It is assumed from research on structurally-related phthalates that DnHP is rapidly absorbed as the monoester from the gut following oral exposure.

Genetic Toxicity. DnHP has tested negative in the Salmonella and two other bacterial assays and in a mouse micronucleus test (*2, 14*). 6,10- Phthalate mixture has tested positive in the mouse lymphoma mutation assay without activation and negative in the Balb/3T3 cell transformation assay (*15*). DIHP was inactive in the mouse micronucleus test (*2*).

Utility of the Data for CERHR Evaluation. There is sufficient data available from a dietary study in rats to show that DnHP can cause liver and thyroid toxicity (1,824 mg/kg bw/day; for 3, 10, or 21 days of exposure). Signs of liver necrosis and slight peroxisomal proliferative changes (histologic and biochemical) were observed. In these same studies, testis weight and gross appearance was unaffected.

Limited dermal toxicokinetic information for DnHP in rodents suggests dermal absorption with renal excretion in 7 days. Kinetic information from structurally related phthalates suggests that DnHP is rapidly absorbed from the gut as the monoester and as n-hexanol after oral exposure.

5.1.3 Developmental Toxicity

Data for DnHP are limited to 1 screening assay (*25*) in which a massive oral dose (9,900 mg/kg bw/day) was administered to 48 mice on gd 6–13 (*16*). None of the 34 pregnant dams gave birth to a live litter. These positive results (lethality) in a screening assay are of relevance to the Panel's evaluation. A reduction in live pups per litter was observed in CD-1 mice exposed to the lowest dose of DnHP (380 mg/kg bw/day) in a reproductive toxicity assay (*18, 21*).

Utility of the Data for CERHR Evaluation. The data from one screening level study in mice, administered one dose level, 9,900 mg/kg bw/day on gd 6–13, are sufficient to indicate that DnHP is a developmental

toxicant at high doses in mice, resulting in prenatal deaths of all litters. This study result provides hazard identification, but it does not provide dose-response information and is therefore inadequate for determination of LOAELs or NOAELs.

5.1.4 Reproductive Toxicity

Reproductive studies for DnHP include a continuous breeding study in mice (*18, 19*) and a 4-day exposure study in rats (*22*). Testicular weights were also measured in a 21-day subchronic exposure study in Wistar rats (*11*). In the one-generation study, male and female mice were exposed to 0, 0.3, 0.6, or 1.2% DnHP in the diet (~0, 380, 800, or 1,670 mg/kg bw/day) throughout a 98-day breeding period (*18, 19, 21*). A NOAEL was not identified because reproductive effects were observed at all dose levels. Fertility was reduced in all treated groups in a dose related manner, with severe reduction at doses of 800 mg/kg bw/day and higher, and complete infertility at the highest dose (1,670 mg/kg bw/day). The number of litters produced and pup survival were reduced in the lowest dose group (380 mg/kg bw/day). Mating of high-dose animals to control animals demonstrated that both males and females were affected. Testicular atrophy and reduced sperm counts were demonstrated in the high-dose males. The high dose group was infertile, the middle-dose and the low-dose groups were subfertile. Thus, a NOAEL was not achieved. A LOAEL of 380 mg/kg bw/day was assessed based on fewer litters and decreased numbers of pups per litter. These mid- and low-dose groups were not evaluated at necropsy, and the lack of a thorough assessment of an unaffected group leads us to state that while our confidence in the quality of the study is high, our confidence is moderate-to-low that these doses correctly represent the true LOAEL.

Results of the mouse study are supported by a subacute study in which testicular atrophy was observed in 4-week-old Sprague Dawley rats gavaged with 2,400 mg/kg bw/day for 4 days (*22*). This study was designed to compare different phthalate esters, rather than to provide dose-response information and is of limited utility for risk assessment. It does show that DnHP is a reproductive toxicant at high dose levels in the young male rat. However, testicular weights were unaffected in 4-week-old Wistar rats fed 1,824 mg/kg bw/day through the diet for 21 days (*11*). The evidence indicates that at oral doses of 380 mg/kg bw/day and higher, DnHP is a reproductive toxicant to male and female mice and male rats. Findings of an *in vitro* assay suggested that testicular toxicity may result in part from primary damage to the Sertoli cells that ultimately leads to the detachment of germ cells (*23*).

Steroid Hormone Activity. An isomeric mixture of dihexyl phthalates exhibited weak activity in an *in vitro* assay that measured binding of phthalates to estrogen receptors (*24*). *In vivo* assays demonstrated that an isomeric mixture of dihexyl phthalates does not increase uterine wet weight or vaginal epithelial cell cornification in immature or mature ovariectomized rats (*24*). The findings suggest that for compounds that may contain DnHP as a component, toxicity is not mediated through estrogenic activity.

Utility of the Data for CERHR Evaluation. The two rat studies and one mouse study are sufficient to indicate that DnHP is a reproductive toxicant to male and female mice and to male rats. The testis is the target organ and damage to the Sertoli cells may be the primary lesion. The mouse study indicates reduced fertility at 800 mg/kg bvw/day and infertility at 1,670 mg/kg bw/day, with reduced numbers of litters and a reduced postnatal survival at 380 mg/kg bw/day (the low dose). No NOAEL was identified in this study. In the short-term (4-day and 21-day) studies in male rats, testicular atrophy occurred at 2,400 mg/kg bw/day by gavage for 4 days, but no effects were noted on testis weight after 21 days at 1,824 mg/kg bw/day in the diet. Consistent male testicular effects were therefore observed in two species. These data do not allow confidence in the assignment of NOAELs and LOAELs for reproductive toxicity in animal models.

5.2 Integrated Evaluation

DnHP is a component in mixtures of 6,10-phthalates (<1%) and diisohexyl phthalates (up to 25%). While some exposure to humans occurs through contact with consumer products, the level is expected to be low. There are no data that document the use of the phthalate mixtures in medical devices. As with all phthalates, intake through diet is expected to be the primary route of exposure; absorption through skin contact is assumed to be negligible. Potential sources of phthalates in foods include migration from packaging materials and general environmental contamination. There are no data on the toxicokinetics of DnHP following exposure by the oral route. However, based on data from other phthalates, it is quite plausible to predict that orally administered DnHP would be converted to the monoester by intestinal enzymes and then rapidly absorbed and excreted.

There are no human data from which to assess the health hazards associated with DnHP exposure. Studies of DnHP toxicity are limited to rats and mice. In the absence of human data, it is assumed that the effects observed in rodents are relevant to humans.

Limited data indicate that the liver is the target organ for adverse effect after exposure to relatively high doses. Such data are generally consistent with effects observed with related phthalates. Limited studies with mixtures that contain DnHP provide little evidence of estrogenic activity.

In a screening protocol design in mice, complete litter loss was observed at a massive oral dose (9,900 mg/kg bw/day). This study is sufficient to determine that DnHP is a developmental toxicant in animals following high exposures, however, it is not sufficient to set a NOAEL. Since only one dose was tested, there is no information on the shape of the dose-response curve. The reduced litter survival observed in the breeding study in mice confirms effects on litter survival and also indicates reduced offspring survival at 380 mg/kg bw/day. A NOAEL was not identified.

Experimental animal data are adequate to establish that DnHP is a reproductive toxicant in rodents. A multiple-oral dose study in mice demonstrated that DnHP treatment induced adverse reproductive effects at the lowest dose tested (380 mg/kg bw/day); the NOAEL is therefore unknown. Infertility occurred in both males and females. Unfortunately, the study did not examine reproductive function in the F_1 offspring. The rodent data are of assumed relevance for humans, but are inadequate to determine dose-response relationships for risk evaluation in humans.

Summary

DnHP is a plasticizer found in 6,10-phthalate substances and DIHP. The phthalate substances are used in numerous consumer products and in some cases, DnHP is added directly to consumer products. In the general population, exposure to DnHP occurs primarily through food. DnHP is poorly absorbed through the skin, but is predicted to be absorbed through the digestive tract. When ingested, it is quite plausible that DnHP would be converted to the monoester by intestinal enzymes and then rapidly absorbed and excreted. The Expert Panel believes that general population exposure to DnHP will not exceed exposure estimates of 3–30 µg/kg bw/day, the estimates derived for DEHP.

Most of the toxicological data were collected from studies in rodents. There are adequate oral studies to indicate that the major targets for effects in rodents are the liver and reproductive tract. In a multiple-dose continuous breeding study in mice, adverse affects on reproduction were observed in the lowest dose group (380 mg/kg bw/day); a NOAEL was therefore not established. The adverse reproductive effects in rodents are assumed to be relevant to potential for human toxicity; however, the data are inadequate for a confident risk assessment.

In a developmental screening study in mice, complete litter loss was noted at the single massive dose administered by gavage (9,900 mg/kg bw/day). Reduced litter survival was also observed in the continuous breeding study in mice at the lowest dose level (380 mg/kg bw/day). The data are inadequate to assess DnHP-induced toxicity to human development.

5.3 Critical Data Needs

Although there is little or no commercial production or use of pure DnHP, it was reviewed to determine structure-activity relationships with other phthalates. DnHP may be present in 6,10--phthalate mixtures (less than 1%) and in diisohexyl phthalate mixtures (up to 25%). Therefore, the public may be better served by focusing on data needs for the diisohexyl phthalate mixtures instead of pure DnHP. Those data needs include:

- Evaluation of exposure to DIHP. If there is exposure, then:
- A perinatal developmental study by the oral route in rats that addresses landmarks of sexual maturation and provides dose-response estimation.
- A toxicokinetic study by the oral route; preferably in the rat, then other species as appropriate.
- Human exposure studies based upon critical exposure periods
- A perinatal developmental study by the oral route in a non-rodent species.

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